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AUTHORITY

BDRL, D/A ltr, 28 Sep 1971

AD

TECHNICAL MANUSCRIPT 370

EFFECT OF UV-IRRADIATED HELPER PHAGE ON TRANSFORMATION AND TRANSFECTION OF ESCHERICHIA COLI BY DNA FROM A TRANSDUCING PHAGE

Lois M. Swaney
Robert A. Altenbern

APRIL 1967

DEPARTMENT OF THE ARMY
Fort Detrick
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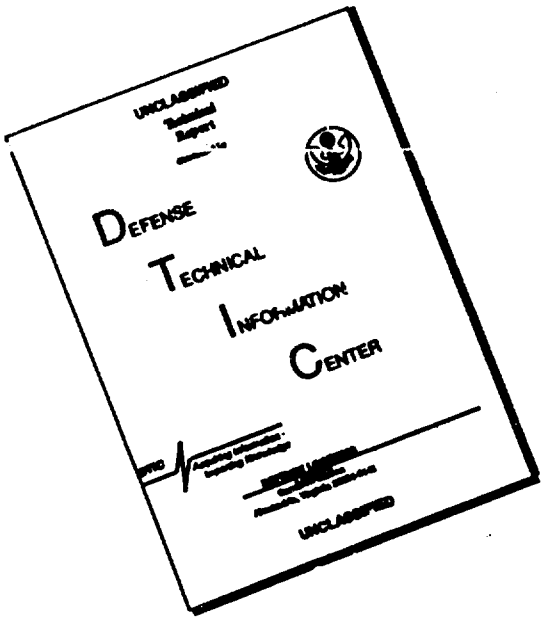

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Project 1C014501B71A

April 1967

EFFECT OF UV-IRRADIATED HELPER PHAGE ON TRANSFORMATION AND TRANSFECTION OF *ESCHERICHIA COLI* BY DNA FROM A TRANSDUCING PHAGE

ABSTRACT

When moderately irradiated helper phage λi^{434} was added to *Escherichia coli* W1485 A23 (*try* A^-) cells and DNA from a non-defective, temperate, transducing hybrid phage carrying bacterial *try* A and B genes ($i^{\lambda}h^{\phi 80}$ *try* A^+B^+), it resulted in an increase in *try*$^+$ transformants and hybrid phage transfectants. The increase may result from prevention of lysis of potential transformants and transfectants because of irradiation damage in the helper phage.

The addition of severely irradiated λi^{434} resulted in a decrease in the number of transfectants but did not reduce the number of transformants. Two possibilities are considered: (i) the decrease in transfectants results from insertion of ultraviolet-light damage in the helper phage DNA into the genome of the hybrid phage, preventing replication of the hybrid phage, and (ii) the registry of the *try* genes carried by the hybrid phage with the bacterial chromosome, resulting in *try*$^+$ transformants, is not affected by insertion of ultraviolet-light damage from the helper phage.

Taylor and Yanofsky[1] reported transformation of a tryptophan A^- mutant of *Escherichia coli* to tryptophan independence when they infected cells with helper phage before adding DNA from a non-defective, temperate, transducing hybrid phage carrying bacterial *try* A and B genes. They suggested some type of physical interaction between the helper DNA and the hybrid DNA in the instances when the helper DNA directed the hybrid DNA to the attachment site of the helper phage on the bacterial chromosome.[2] The hybrid DNA normally attaches at another site.[3]

This communication reports the effect of ultraviolet-light-inactivated helper phage λi^{434} on the recovery of *try*$^+$ transformants and on the simultaneous recovery of hybrid phage transfectants. Transfection, a term suggested by Földes and Trautner,[4] refers to the production of complete virus in cells infected with isolated nucleic acid from that virus.

E. coli K12 strain W1485 A23 (try A^- mutant), non-lysogenic or lysogenic for the hybrid phage $i^{\lambda}h\emptyset80$ try A^+B^+ or Ø80, and Shaw W3101 lysogenic for the helper phage λi^{434} (a λ-434 hybrid with the immunity of 434) were obtained from Dr. Milton W. Taylor. Strain W1485 wild type was obtained from Dr. Esther M. Lederberg, and C600 lysogenic for λ from Dr. Benjamin J. Barnhart.

The procedures of Taylor and Yanofsky[1] were followed with these exceptions. Lysogenic strains were induced with mitomycin C,* and the resultant phages were propagated on W1485. Lysates containing either the helper or the hybrid phage were subjected to several cycles of low- and high-speed centrifugation in 0.01 M Tris + 0.01 M $MgSO_4$, pH 7.8, to concentrate and purify the phage. Plaque-forming units (PFU) of the helper phage were assayed on lawns of W1485 or W1485 A23, and those of the hybrid phage on W3101 (λi^{434}). Try^+ transformant colonies on minimal agar were counted after 48 hours at 37 C. Preparations of hybrid phage DNA were heated to 72 C for 10 minutes and quickly chilled in ice water as described by Barnhart.[4] DNA was used in a final concentration of 10 μg per ml. Suspensions of the helper phage were irradiated 36 cm below a 15-watt "germicidal" lamp** in containers placed on a rotary shaker. Irradiated and non-irradiated phage were added to cells in the ratio of 2.6 phage particles per cell, as determined from the titer of non-irradiated phage.

Table 1 reveals the effect on the transformation system of helper phage exposed for increasing periods to ultraviolet (UV) light. The table also shows the effect of irradiation on the viability of the helper phage. The UV irradiation that reduced the titer of the helper phage by three logs resulted in an increase in try^+ transformants and transfectants. Exposure of the helper phage to more UV light reduced the numbers of transfectants but did not affect the formation of try^+ transformants.

Control experiments (not reported here) showed that infection of W1485 A23 with non-irradiated helper phage resulted in the lysis of 85% of the cells. Increased recovery of both transformants and transfectants among cells infected with moderately damaged helper phage may result from prevention of lysis of potential transformants and transfectants by the helper phage.

The helper phage is a λ phage lacking only the λ immunity region[5] and the hybrid phage contains the λ immunity region and the host range of Ø80.[1] Thus, there must be considerable genetic homology between the genomes of the two phages. Decreased recovery of transfectants that occurs following infection with severely irradiated helper phage may result from insertion of the UV damage in the helper-phage DNA into the genome of the hybrid phage,[6] preventing replication of the hybrid phage.

* CalBiochem, Los Angeles, Calif.
** General Electric Co.

TABLE 1. TRANSFORMATION AND TRANSFECTION OF E. COLI W1485 A23 BY HYBRID PHAGE DNA AFTER TREATMENT WITH IRRADIATED HELPER PHAGE[a/]

Helper Phage		Number per ml of Mixture[b/]	
Minutes Irradiated	PFU Surviving	Transfectants	Transformants
0.5	1.1×10^8	6.9×10^4	275
1.0	3.1×10^8	$>5.0 \times 10^5$	420
2.0	3.1×10^5	1.0×10^5	620
4.0	1.0×10^3	1.1×10^4	790
8.0	3.0×10^2	3.5×10^3	755
None[c/]	2.6×10^9	1.5×10^4	60

a. Each ml of incubation mixture contained 1.0×10^9 cells, 2.6×10^9 phage particles, and 10 μg DNA.
b. Each value is an average of two determinations.
c. A replicate mixture, from which hybrid phage DNA was omitted, contained no try^+ or transfected cells.

Transformation to tryptophan independence was not diminished in the presence of severely irradiated helper phage. Apparently, the hybrid phage, although unable to replicate because of incorporation of damage from the helper phage, may register with the chromosome of the recipient cell and, if so, may subsequently transform from try^- to try^+. The portion of the hybrid phage DNA carrying the try A^+B^+ genes presumably has no homology with the damaged helper phage.

Transformant colonies obtained with non-irradiated helper phage were reported to be lysogenic for λi^{434} and the hybrid phages[1] and to be smooth- or rough-edged.[2] This colonial morphology was shown to be associated with the chromosomal location of the hybrid phage.[2] Our studies included an examination of progeny of transformant colonies obtained with irradiated helper phage. Many try^+ progeny of smooth-edged colonies were non-lysogenic and sensitive to λ, λi^{434}, the hybrid, and ø80 phages, but others were lysogenic for the hybrid phage. Assessment of the lysogenic characteristics of progeny of rough-edged colonies was inconclusive because of the segregation of try^- cells, also noted by Taylor and Yanofsky,[2] and the large numbers of free hybrid phage in the transformed clones discovered in the course of these experiments. Progeny that were examined were not lysogenic for λi^{434}, although they did harbor the hybrid phage.

Goldschmidt and Landman[7] demonstrated transduction of Salmonella typhimurium LT-2 by high multiplicities of UV-inactivated virulent PLT-2 bacteriophage that resulted in the recovery of phage-sensitive, non-lysogenic transductants. The UV treatment of helper phage in the transformation system described in this paper provides a method for increasing the transformation rate to try independence, possibly by preventing lysis of potential transformants by the helper phage, and may provide a method for studying transformation without lysogenization.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)

Department of the Army
Fort Detrick, Frederick, Maryland 21701

2a REPORT SECURITY CLASSIFICATION
Unclassified

2b GROUP

3 REPORT TITLE

EFFECT OF UV-IRRADIATED HELPER PHAGE ON TRANSFORMATION AND TRANSFECTION OF ESCHERICHIA COLI BY DNA FROM A TRANSDUCING PHAGE

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

5 AUTHOR(S) (Last name, first name, initial)

Swaney, Lois M.
Altenbern, Robert A.

6 REPORT DATE	7a TOTAL NO. OF PAGES	7b NO. OF REFS
April 1967	10	7

8a CONTRACT OR GRANT NO.	9a ORIGINATOR'S REPORT NUMBER(S)
b PROJECT NO. 1C014501B71A	Technical Manuscript 370
c	9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d	

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14. Key Words

Escherichia coli
DNA
Bacteriophage
Transformation
Transduction

DD FORM 1 JAN 64 1473

Unclassified
Security Classification